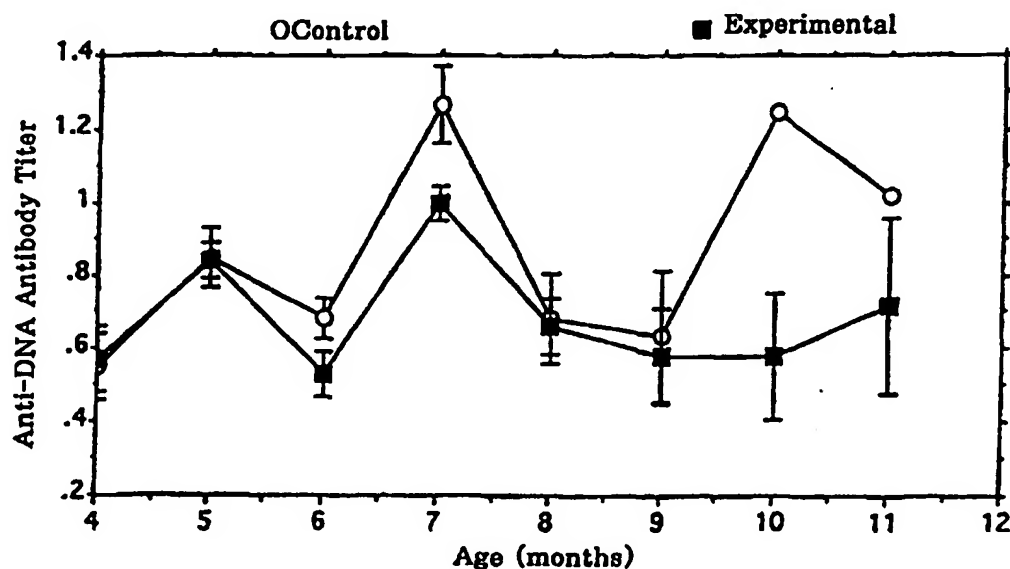




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(54) Title: PACLITAXEL FOR THE TREATMENT OF RHEUMATIC DISEASES



(57) Abstract

Paclitaxel has been found to have a potential effect on the protection against the development of rheumatic disease. Therefore, paclitaxel is supposed to be a potential agent for the treatment of rheumatic diseases, especially systemic lupus erythematosus (SLE).

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PACLITAXEL FOR THE TREATMENT OF RHEUMATIC DISEASES

BACKGROUND OF THE INVENTION

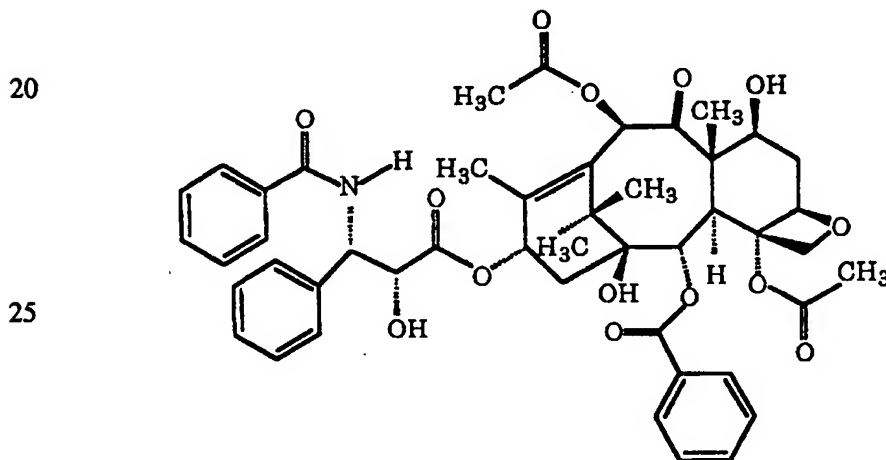
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1. Field of the Invention

The present invention relates to a novel use of paclitaxel for the treatment of rheumatic diseases. More particularly, the invention relates to the use of paclitaxel
10 for the treatment of systemic lupus erythematosus (SLE).

2. Description of the Prior Art

Paclitaxel belongs to the taxane family of compounds. It has the chemical
15 name of $5\beta,20$ -epoxy- $1,2\alpha,4,7\beta,10\beta,13\alpha$ -hexahydroxytax- 11 -en- 9 -one $4,10$ -diacetate
 2 -benzoate 13 -ester with $(2R,3S)$ - N -benzoyl- 3 -phenylisoserine and the following
structural formula:



30 Paclitaxel, which is commercially available under the trade name of TAXOL, has been known and used as an anti-cancer agent, especially for ovarian and breast cancers. The cytotoxic effects of this compound is based on its unique mechanism which promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This leads to disruption of normal
35 microtubule dynamics required for cell division and vital interphase processes which culminate in cell death: See Schiff P. B. *et al.*, 1979, "Promotion of microtubule

assembly *in vitro* by taxol," 1979, *Nature* 277:665-667; and Rowinsky E. K. *et al.*, "Paclitaxel," 1995, *New England Journal of Medicine* 332(15):1004-1014.

Paclitaxel has also been shown to enhance interleukin- β and tumor necrosis factor- α (TNF- α) production. In relation to this property of paclitaxel, several reports indicate the role of TNF- α in the development of autoimmune diseases and the replacement therapy for the treatment of the diseases. Jacob C. O. *et al.* presented evidence that TNF- α could be involved in the pathogenesis of lupus nephritis in (NZB X NZW) F₁ mice, an animal model of autoimmune disease in "Tumor necrosis factor- α in murine autoimmune lupus nephritis," 1988, *Nature*, 331(28):356-137. Hiroshi Ishida *et al.* suggested that the protection by the anti-interleukin 10 antibodies against autoimmunity is due to an anti-IL-10-induced upregulation of endogenous TNF- α in "Continuous administration of anti-interleukin 10 antibodies delays the onset of autoimmunity in NZB/W F₁ mice," 1994, *J. Exp. Med.* 179:305-310.

15

Paclitaxel has also been found to completely preclude the development of rheumatoid arthritis and also suppress the established clinical disease in an animal model: See Brahn E. *et al.* "Regression of collagen induced arthritis with taxol, a microtubule stabilizer," 1994, *Arthritis Rheum.*, 37(6):839-845. This effect was shown to be augmented further by the addition of an angiogenesis inhibitor: See Oliver S. J. *et al.*, "Suppression of collagen-induced arthritis using an angiogenesis inhibitor, AGM-1470, and a microtubule stabilizer, taxol," *Cellular Immunology*, 157:291-299. The mechanism of action of these agents was postulated to be multifold including inhibition of mitosis and chemotaxis of the inflammatory cells, inhibition of antigen processing and presentation by the macrophage, inhibition of angiogenesis and so forth, which are core mechanisms of pathogenesis of many autoimmune diseases.

However, none of the studies and reports described above provide or suggest any possibility of paclitaxel being successfully used to manage and treat rheumatic diseases including systemic lupus erythematosus (SLE).

The inventors of the present application have studied on the effect of paclitaxel in the treatment of rheumatic disease based on the several characteristic mechanisms of rheumatic disease pathogenesis and found that paclitaxel improved the clinical

course of murine lupus after development of autoimmunity. Although it is uncertain at present whether changes in TNF- α levels or effects on microtubule polymerization, or both provided the most benefit in the case concerned, paclitaxel improved the survival rate of the animals which is undoubtedly due to the decreased anti-dsDNA
5 antibody titer and the blood urea nitrogen (BUN).

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a paclitaxel
10 preparation which has a potential effect in the treatment of rheumatic disease. The paclitaxel preparation is particularly effective for the treatment of systemic lupus erythematosus (SLE) which is found to have the highest mortality among systemic rheumatic diseases.

15 Further objects and advantages of the invention will become apparent through reading the remainder of the specification.

BRIEF DESCRIPTION OF THE DRAWINGS

20 Figure 1 is a graph showing the survival rates of paclitaxel treated and control groups of mice as a function of the duration of the treatment.

Figure 2 is a graph showing the change of the blood urea nitrogen (BUN) as a function of the duration of the treatment.

25

Figure 3 is a graph showing the change of proteinuria as a function of the duration of the treatment.

Figure 4 is a graph showing the change of serum anti-dsDNA antibody titer
30 as a function of the duration of the treatment.

DETAILED DESCRIPTION OF THE INVENTION

The potential effect of paclitaxel on the protection against the development of
35 rheumatic diseases, especially systemic lupus erythematosus (SLE), was proved using (NZB X NZW) F₁ hybrids, an animal model of systemic lupus erythematosus in

humans.

Systemic lupus erythematosus (SLE) is a chronic and systemic kind of rheumatic diseases which primarily attack the tissue and blood system including
5 connective tissue. Although the pathogenesis of this disease is not yet fully known, many studies suggest that it is a disease of an autoimmune reaction which is generated by an antibody to its own tissue.

While eleven standard examinations for the diagnosis of SLE are established
10 by the American Rheumatism Association, several basic diagnostic examinations are generally conducted: for example, a blood examination in general including measurement of the erythrocyte, leucocyte and platelet counts, urinalysis (measurement of proteinuria), examinations for kidney function and chest X-rays and cardiac ultrasound examinations.

15

Among these diagnostic methods, serum anti-dsDNA antibody titer, complement concentration in the blood, proteinuria, serum creatinine concentration, erythrocyte sedimentation rate, and leucocyte and platelet counts are important and useful for the observation of the clinical course and the anticipated activity of the
20 disease. Protein in urine (proteinuria) indicates an attack into the kidney and varies depending on the activity of the disease. Blood urine nitrogen (BUN) is also an indication of kidney function. An increase in BUN implies deterioration of kidney function while a decrease in BUN implies its improvement. Thus, BUN is a very useful tool for evaluating the reaction to a treatment agent. Finally, anti-dsDNA
25 antibody is the most specific antibody which can be found in SLE cases. Thus, it could be a definite indication for the diagnosis of the disease and the effect of the treatment agent as well.

For the clinical use according to the present invention, paclitaxel can be
30 formulated into a parenteral preparation. A solution for injection is particularly preferred. A paclitaxel solution preparation can be prepared, for example, by dissolving paclitaxel in a pharmaceutically acceptable solvent in an appropriate concentration (preferably 0.1 to 10%) and, if necessary, with stabilizers and/or buffering solutions. Paclitaxel solution for injection can be distributed in ampules or
35 vials with different doses. Alternatively, paclitaxel can be formulated into a parenteral dry preparation which can be reconstituted with a suitable solvent before

use.

Dosage of paclitaxel can be varied in a wide range depending on the type and severity of disease, administration route and individual requirements of a patient. For intravenous injections, an effective daily dosage is 7.5mg/m² to 15mg/m² of body surface area. The daily dosage is generally administered over a five day period in a week and this administration pattern can be repeated for four weeks, and possibly for 6 to 12 months depending on the severity of the disease.

In lethality studies, Sprague-Dawley rats were intraperitoneally administered a paclitaxel injection solution once a day for 1 day or once a day for 5 days, while CD2F₁ mice were treated once a day for 5 days. Doses lethal to 10%, 50%, and 90% (LD₁₀, LD₅₀, and LD₉₀, respectively) of the rats given a single dose were 138, 206, and 307 mg/m², respectively. The LD₁₀, LD₅₀, and LD₉₀ for rats treated once a day for 5 days were 36, 51 and 74 mg/m² per day, respectively, whereas corresponding values in mice were 70, 82 and 97 mg/m² per day.

To address the effect of paclitaxel on the severity of the autoimmunity, we carried out a series of experiments as follows.

20

1. Animal Model

New Zealand Black X New Zealand White (NZB X NZW) F₁ female mice were purchased from Harlan-Sprague Dawley. This strain is known to develop a severe autoimmune disease culminating in death from nephritis by 1 year of age: See, Theofilopoulos A. N. *et al.*, "Murine models of systemic lupus erythematosus," 1985, Advanced Immunology 37:269. These models develop some immunological features similar to systemic lupus erythematosus in humans which includes antibodies to dsDNA antigens and the subsequent development of immune complex-mediated glomerulonephritis. Treatment was started when the mice reached 5 months of age.

30

2. Treatment with Paclitaxel

A paclitaxel sample, TAXOL was purchased from Bristol-Myers Squibb, Princeton, New Jersey. Taxol was initially solubilized in a 1:1 dilution of ethanol and Cremophor EL (Sigma). Normal saline was added to make a final concentration

35

of 2.4 mg/ml taxol in 5%(w/v) ethanol and 5%(w/v) Cremophor EL, prior to administration by intraperitoneal injection. A total of 35 mice were divided into two groups; one group of 15 mice was a treatment group and the other group of twenty mice was the control group. The treatment group was injected intraperitoneally with the taxol solution at a dose of 10mg/kg every other day for 6 days, and this treatment was followed by the same route and pattern of administration except that the dose was reduced to 7.5mg/kg. This two-step administration pattern was repeated every fourth week for 6 months. For the purpose of comparison, the same volume of vehicle containing 5% ethanol and 5% Cremophor in normal saline was administered to the control group of 20 mice in the same manner.

3. Assessment

Survival rate: Survival rates in each group were calculated by the Kaplan Meier method for individual periods of treatment through out followup and compared by the log-rank test.

Renal disease: The development of renal disease was assessed by the appearance and level of the proteinuria and by elevation of the BUN. Proteinuria was measured colorimetrically using dipsticks (Albustix; Miles laboratories, Inc., Elkhart, IN) and was graded as follows: trace, 10mg/dl; 1+, 30mg/dl; 2+, 100mg/dl; 3+, 300mg/dl; and 4+, 1,000mg/dl. BUN was measured by azostix(Milas Laboratories).

Anti-dsDNA antibody : Serum antibodies specific for double stranded DNA were quantified by ELISA as described in Zouali M., B. D. Stollar, 1986, "A rapid ELISA for measurement of antibodies to nucleic acids antigens using UV-treated polystyrene microlates," J. Immunol. Methods. 90:105.

Fluorescence analysis of lymphocytes subpopulations: Peripheral blood was obtained from individual mice by retroorbital bleeding into heparinized pipets. Mononuclear cells were separated by centrifugation over Lymphocyte-M (Cedarlane Laboratories, Ontario, Canada) and quantified using a Coulter ZBI cell counter. Mononuclear subsets were identified by staining with fluorescein-conjugated monoclonal antibody (mAb), as described in Seaman W. E. *et al.*, "Treatment of autoimmune MRL lpr/lpr mice with monoclonal antibody to Thy-1.2: a single injection sustained effects on lymphoproliferation and renal diseases," Journal

Immunology 130:1713. The antibodies used for analysis included: anti-CD4 to identify helper T cell subsets, CD8 to identify suppressed T cell subsets and B220. Fluorescein isothiocyanate(FITC) conjugated rat anti-mouse CD4 and B220 mAb, phycoerythrin(PE) conjugated rat anti-mouse CD8 mAb were used to detect anti-CD4
5 on target cell surfaces. Fluorescein analysis was performed immediately prior to the initiation of the treatment.

Statistical analysis: Means, standard deviation (SD) and standard error of mean (SEM) were calculated by standard method. A one factor analysis of variance
10 (ANOVA) for repeated measures was used for continuous variables for significance of differences between the two groups. Results were considered to be at the confidence level of 90% or higher.

4. Results and Discussions

15

As can be seen from Figure 1, 80% of the vehicle treated mice died between 38 and 144 days and the survival pattern did not differ from that of untreated control mice as reported previously. On the other hand, half of the paclitaxel treated mice were alive after 160 days of observation ($p=0.04$ by log-rank test). There was no
20 evidence of hypersensitivity reaction in either of the groups. Thus, the administration of paclitaxel prolonged the survival of the (NZB X NZW) F_1 mice.

In Figure 2, while the degree of proteinuria appeared to be less in the experimental group, the difference reached only the 82% confidence level by
25 ANOVA for repeated measurement which is not so significant. BUN was also significantly diminished in paclitaxel treated group compared to the vehicle treated animals(Figure 3). This implies that kidney function has been improved by the treatment with paclitaxel. Thus, the diminution in the impairment of renal function as reflected by the serum BUN may very likely have been the cause for the increased
30 survival of the paclitaxel treated (NZB X NZW) F_1 mice.

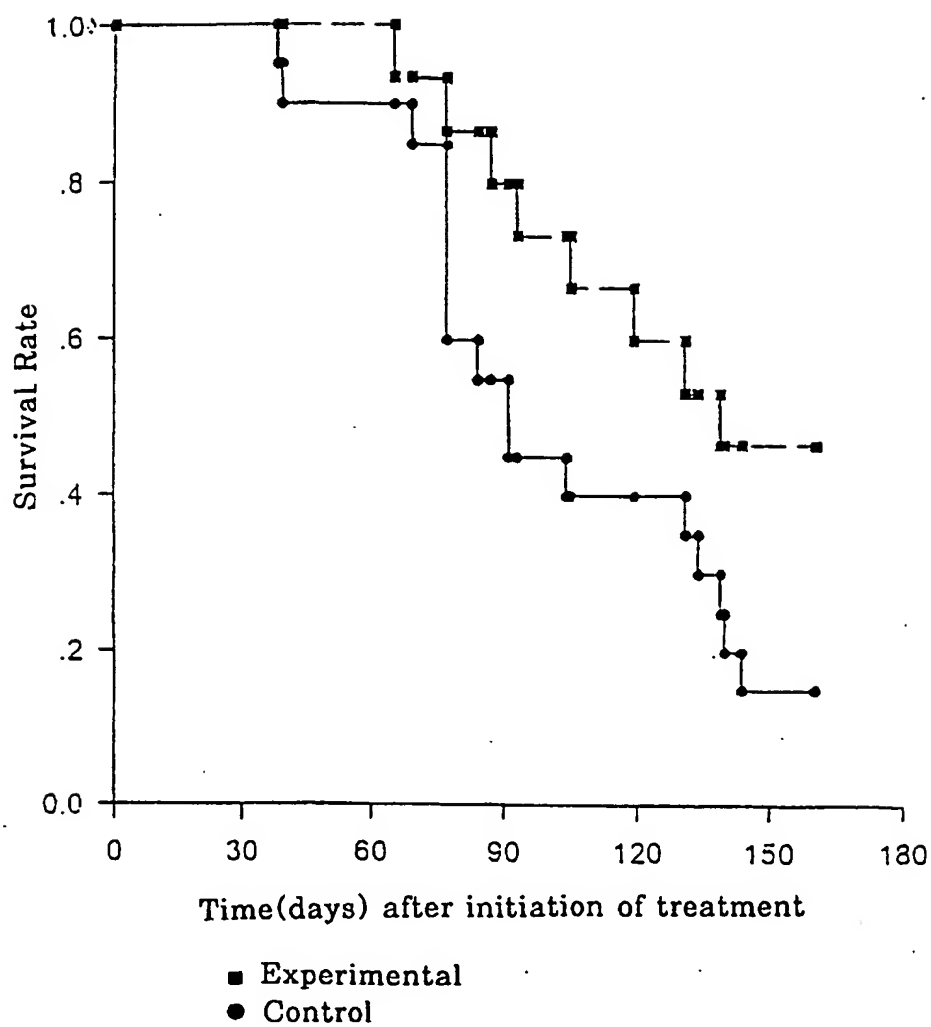
In Figure 4, anti-dsDNA antibody titers were significantly diminished in paclitaxel treated group compared to the vehicle treated animals. This also demonstrates that the clinical course of SLE could be improved by paclitaxel
35 treatment.

What Is Claimed Is:

1. A preparation for treatment of rheumatic diseases comprising an effective dosage of paclitaxel and suitable excipients.
- 5 2. The preparation of Claim 1 wherein said rheumatic disease is systemic lupus erythematosus.
3. The preparation of Claim 1 which is a solution for injection.
- 10 4. The preparation of Claim 1 comprising paclitaxel in a concentration of 0.1 to 10%(w/v).

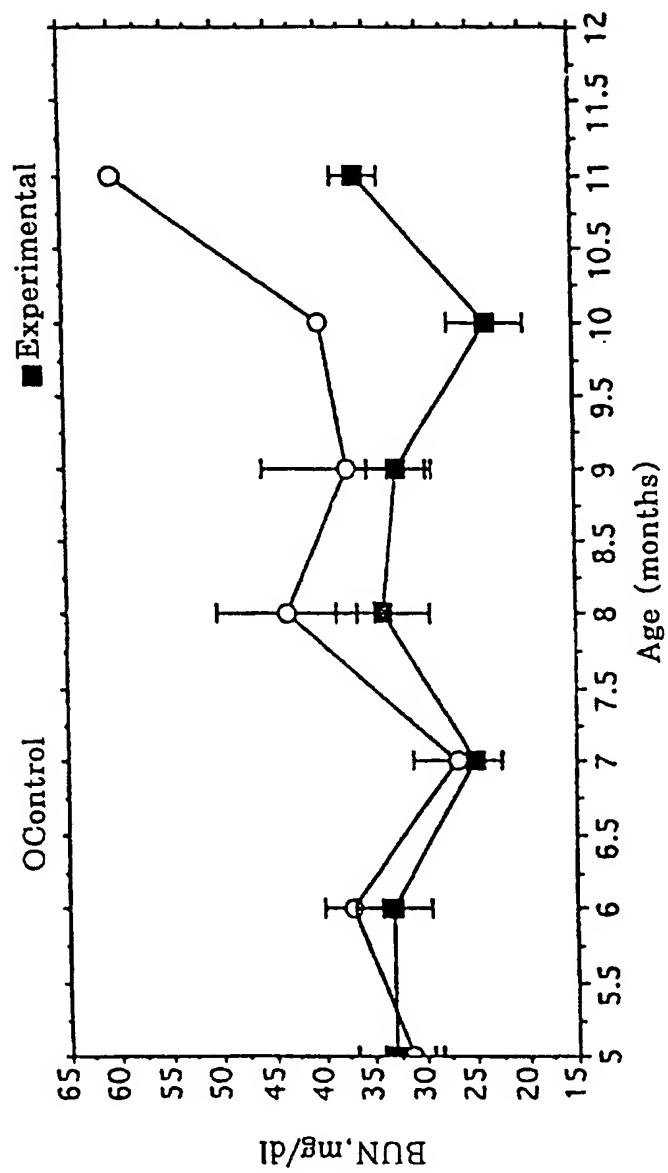
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FIG. 1



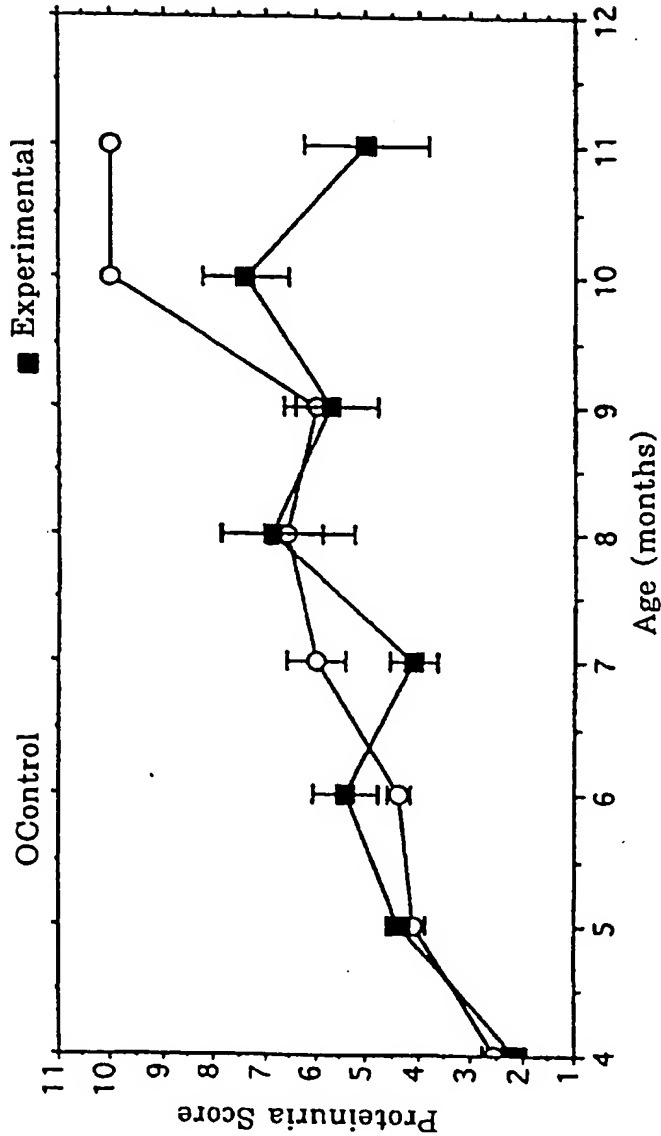
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FIG. 2



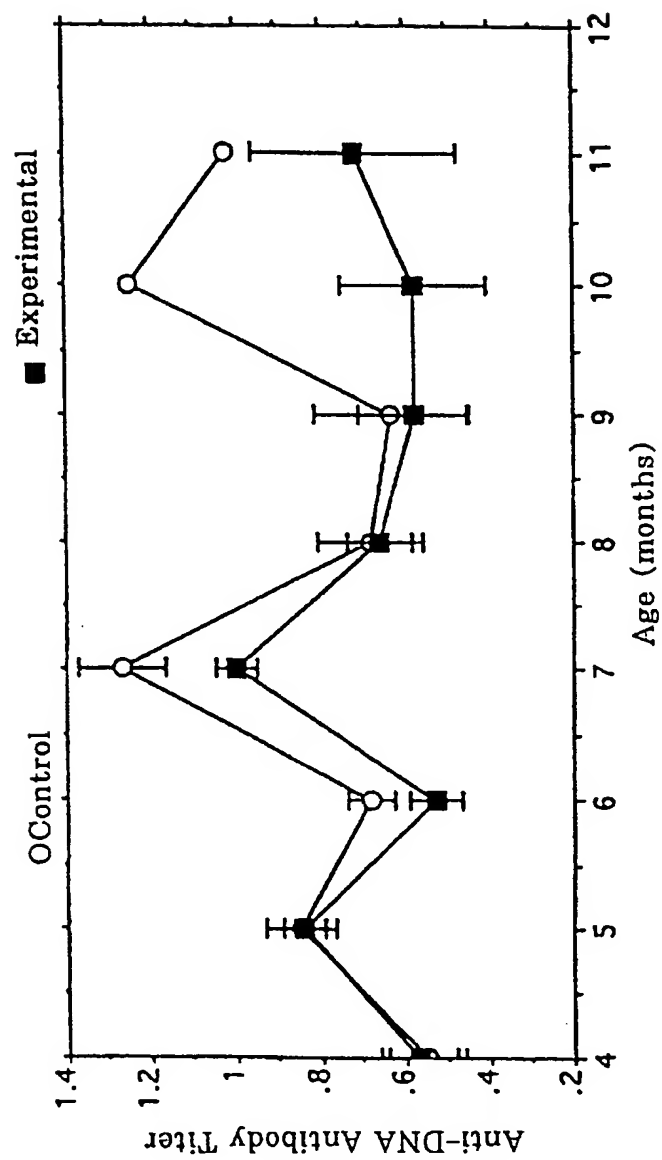
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FIG. 3



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FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR 96/00183

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁶: A 61 K 31/335

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁶: A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Questel (FI CAS, FI WPIL)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/00 928 A1 (RHONE-POULENC RORER S.A.) 21 January 1993 (21.01.93), abstract, claims 1,4-7.	1-4
X	WO 94/12 171 A1 (RHONE-POULENC RORER S.A.) 09 June 1994 (09.06.94), abstract; claims 1,2,4-7,9.	1-4
X	WO 94/12 172 A1 (THOMAS JEFFERSON UNIVERSITY) 09 June 1994 (09.06.94), abstract; claims 1,7,10; page 9, line 15 - page 10, line 20.	1-4
X	WO 94/12 198 A1 (F.H.FAULDING & CO. LTD.) 09 June 1994 (09.06.94), abstract; claims 1,2,8,12.	1-4
X	Chemical Abstracts, Vol.106, No.22, 01 June 1987 (Columbus, Ohio, USA), page 405, column 1, abstract No.182581c, B.D. TARR et al.: "A new parenteral vehicle for the administration of some poorly water soluble anti-cancer drugs", & J. PARENTER. SCI. TECHNOL., 41(1), 31-3 (Eng).	1-4
A	BRAHN e. et al.: "Regression of Collagen-Induced Arthritis with Taxol, a microtubule Stabilizer"; Arthritis & Rheumatism, June 1994, Volume 37, No.36,	1-4

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>pp. 839-845, abstract, (cited in the application).</p> <p>OLIVER S.J. et al.: "Suppression of Collagen-Induced Arthritis Using an Angiogenesis Inhibitor, AGM-1470, and a Microtubule Stabilizer, Taxol"; Cellular Immunology, August 1994, Volume 157, No.1, pp. 291-299, abstract, (cited in the application).</p> <p>-----</p>	1-4

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